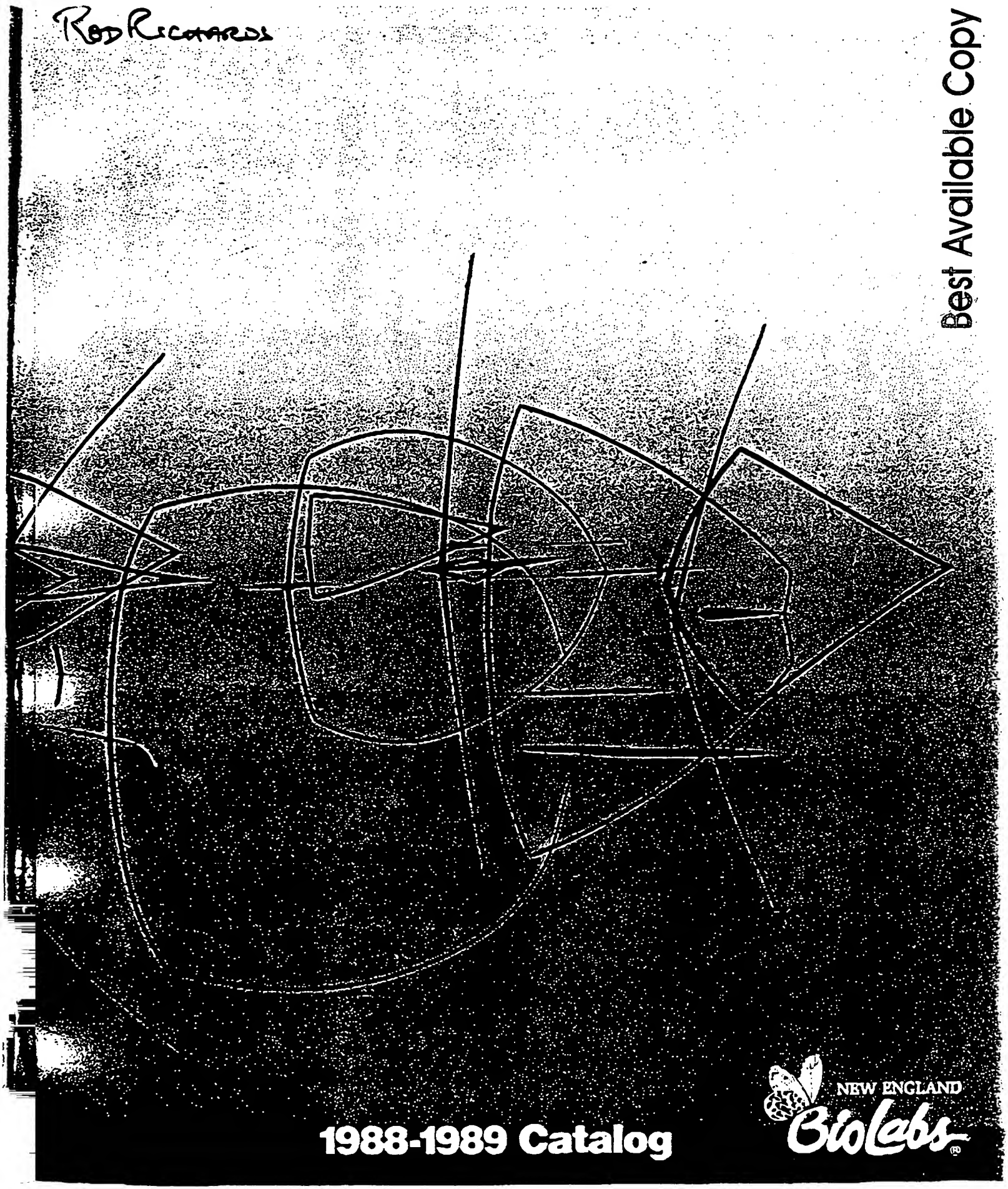
EXHIBIT A

Rod Richards



1988-1989 Catalog

NEW ENGLAND BioLabs®

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T4 DNA Ligase
#202

20,000 units $55
100,000 units $220

#202-C
(Highly Concentrated 2,000,000 units/ml)

20,000 units $55
100,000 units $220

Description: Catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA. This enzyme will join both blunt-ended and cohesive-ended restriction fragments of duplex DNA. The enzyme is purified from *E. coli* C600 pcI857 pPLc28lig 8(1). Contains no detectable exonuclease and endonuclease activity.

Assay Conditions: 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP, 50 μg/ml bovine serum albumin and DNA (0.1 to 1 μm in 5' termini). Incubations are at 16°.

Unit Definitions: One unit is defined as the amount required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16° in 20 μl of the above assay mixture and a 5' DNA termini concentration of 0.12 μM (300 μg/ml). The example shown indicates a concentration of 4.0×10^6 units/ml.

Blunt-end Ligation: In general about 50 times as much enzyme is required to achieve the same extent of ligation for blunt-ended as cohesive-ended DNA fragments. Although ligation of blunt-ended DNA fragments requires higher levels of T4 DNA ligase, the concentration of ligase offered by New England Biolabs is more than adequate to achieve greater than 95% ligation of blunt-ended DNA fragments in a short period of time (see figures). Furthermore, the product of the ligase reaction can be fully re-cleaved by the restriction endonuclease which generated the blunt-ended fragments, indicating that the termini are left intact.

Relationship to Other Ligase Units: One cohesive end ligation unit (defined above) equals:
a) 0.015 ATP-PP exchange unit (2)
b) 0.0025 d(A-T) circle formation unit (3)

Transformation Efficiency After Ligation: In order to evaluate the T4 DNA ligase under ligation conditions used in cloning experiments, the following procedure is performed with the ligase: *E. coli* strain JM101 is transformed with the M13mp8 listed below. Where indicated, the M13mp8 RF DNA is cleaved with *Eco*R I and ligated at the concentration of 4 μg DNA per ml of reaction.

M13mp8 DNA	% Transformants	Ratio colorless/blue plaques
Supercoiled	100%	< 0.1%
*Eco*R I cleaved	0.5%	< 1.0%
*Eco*R I cleaved and ligated	20–40%	< 0.1%

The *Eco*R I site of M13mp8 is within the gene encoding beta-galactosidase. Any damage which occurs at the site of ligation and results in a transformant is easily detected on color indicator plates for beta-galactosidase activity. The ratio of colorless/blue plaques indicates the relative number of transformants due to damage at the cleavage site.

Enzyme Purity: T4 DNA ligase is run in an SDS polyacrylamide gel system. Each preparation of enzyme is at least 99% pure as indicated by relative band intensities.

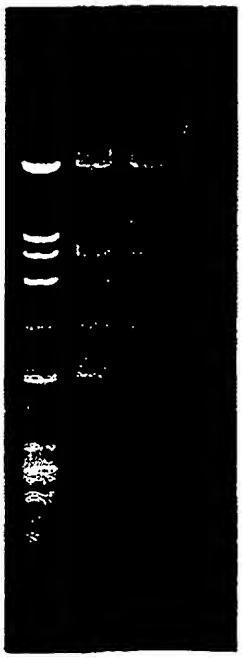
Concentration and Shipping: 100,000 to 500,000 units/ml. Supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 μg/ml bovine serum albumin and 50% glycerol. Store at −20°.

Reference: (1) Remaut, E. and Fiers, W., unpublished observations
(2) Weiss, B., Jacquimin-Sablon, A., Live, T.R., Fareed, G.C. and Richardson, C.C. (1968) *J. Biol. Chem 243*, 4543–4555
(3) Modrich, P. and Lehman, I.R. (1970) *J. Biol. Chem. 245*, 3626–3631

Ligation of *Hind* III fragments of Lambda DNA using 1 μl T4 DNA Ligase at a 1:400 dilution

0' 10' 20' 30' 60'

Ligation of *Hae* III fragments of Lambda DNA using various amounts of T4 DNA ligase in a 20 μl reaction volume incubated for 30 minutes at 16°.



0 0.1 0.2 1.0μl
T4 DNA Ligase

T4 RNA Ligase
#204

1,000 units $44
5,000 units $176

Description: Catalyzes the ATP-dependent ligation of a 5′ phosphoryl-terminated nucleic acid donor to a 3′ hydroxyl-terminated nucleic acid acceptor. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates (1). By optimizing reaction conditions, ligation of single-stranded oligodeoxyribonucleotides of up to 40 bases in length are possible (2). Purified to over 90% homogeneity from *E. coli* RRI containing the plasmid pRF-E35 that overproduces T4 RNA Ligase [constructed at New England Biolabs, Inc. after the method of K.N. Rand and M.J. Gait (3,4)]. Free of detectable levels of single-stranded DNA exonuclease, endonuclease, ribonuclease, and phosphatase.

Assay Conditions: 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 10 μg/ml bovine serum albumin, 20 mM dithiothreitol, 1 mM ATP, 10 μM (5′-^{32}P)rA_{20} (10 μM in 5′ termini) and 0.1 to 0.6 units of enzyme. After incubation at 37°, the reaction is terminated by boiling for 2 minutes, and the bacterial alkaline phosphatase-resistant 5′-phosphoryl termini are determined as described (5).

Unit Definition: One unit is defined as the amount required to convert 1 nmole of 5′-phosphoryl termini in (5′-^{32}P)rA_{20} to a phosphatase-resistant form in 30 minutes at 37° (1) at a 5′-termini concentration of 10 μM (6).

Concentration and Shipping: 2,000 to 10,000 units/ml. Supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 μg/ml bovine serum albumin and 50% glycerol. Store at −20°.

Reference: (1) England, T., Gumport, R. and Uhlenbeck, O. (1977) *Proc. Nat. Acad. Sci. U.S.A. 74*, 4839–4842
(2) Tessier, D.C., Brousseau, R. and Vernet, T. (1986) *Anal. Biochem.* 158, 171–178
(3) Rand, K.N., and Gait, M.J. (1984) *EMBO Journal* vol. 3 no. 2, 397–402
(4) Strain constructed by Feehery, R.
(5) Sugino, A., Snopek, T.J. and Cozzarelli, N.R. (1977) *J. Biol. Chem. 252*, 1732–1738
(6) Sugino, A., Goodman, H.M., Heynecker, H.L., Shine, J., Boyer, H.W. and Cozzarelli, N.R. (1977) *J. Biol. Chem. 252*, 3987–3994

DNA Ligase (*E. coli*, NAD)
#205

200 units $44
1,000 units $176

Description: Catalyzes the formation of a phosphodiester bond between juxtaposed 5′ phosphate and 3′ hydroxyl termini in duplex DNA containing cohesive ends. Required NAD (Nicotinamide adenine dinucleotide) for activity. Purified from *E. coli* strain 594 (su$^-$) carrying the prophage λgt4-lop-11 lig$^-$ *Sam* 7 (1) by the procedure of Panasenko et al. (2).

Assay Conditions: 30 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 0.1 mM EDTA, 20 mM dithiothreitol, 26 μM NAD, 50 μg/ml bovine serum albumin and *Hind* III fragments of lambda DNA (300 to 600 μg/ml). Incubations are at 16°.

Unit Definition: One unit is defined as the amount required to give 50% ligation of *Hind* III fragments of lambda DNA in 30 minutes at 16° in 20 μl of the above assay mixture and a 5′ DNA termini concentration of 0.12 μM (300 μg/ml).

Concentration and Shipping: 1,000 to 4,000 units/ml. Supplied in 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 μg/ml bovine serum albumin and 50% glycerol. Stable for many months when stored at −20°.

Reference: (12) Panasenko, S.M., Cameron, J.R., Davis, R.W. and Lehman, I.R. (1977) *Science 196*, 188–189
(2) Panasenko, S.M., Alazard, R.J. and Lehman, I.R. (1978) *J. Biol. Chem. 253*, 4590–4592